Microfabricated Polymeric Vessel Mimetics for Cancer Cell Culture

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Abstract

In chemotherapeutic studies of cancer cells, limitations of current culturing methods can obscure results and hinder drug development. Three dimensional (3D) cell culture offers a more desirable approach that better simulates in vivo physiologic conditions. In vitro 3D tissue models have the capability to facilitate development and screening of cancer therapeutics, but are currently limited by their lack of vasculature and mass transport of nutrients and waste. The purpose of this study was to develop a method for vascularizing 3D cell cultures. This began with the development of a bioreactor system to provide precise control of dissolved oxygen, nutrients, and pH level. To mimic the physiologic perfusion of oxygen from blood vessels, micropillars of photopolymerized silicone hydrogel membranes were microfabricated using photolithography for seeding with ovarian tumor cells. Under this engineered microenvironment we expect to observe different growth patterns and pharmacologic behavior of tumor cells.

Background & Introduction

- Studies evaluating potential cancer therapeutics have been established on in vitro two-dimensional cell culture and in vivo animal models. However, neither method adequately recapitulates the morphology of human tissue and tumorigenesis.[1,2]
- Physiological constraints of current culture methods can alter results of chemotherapeutic studies, thereby increasing drug development costs. By engineering a 3D microenvironment, complex biological processes involved in tumorigenesis and cancer multidrug resistance can be discovered.
- Cell morphology, migration and signaling in 3D microenvironments better mimic physiological conditions compared to routine 2D cell culture. Differences in patterns of gene expression have also been observed[1].

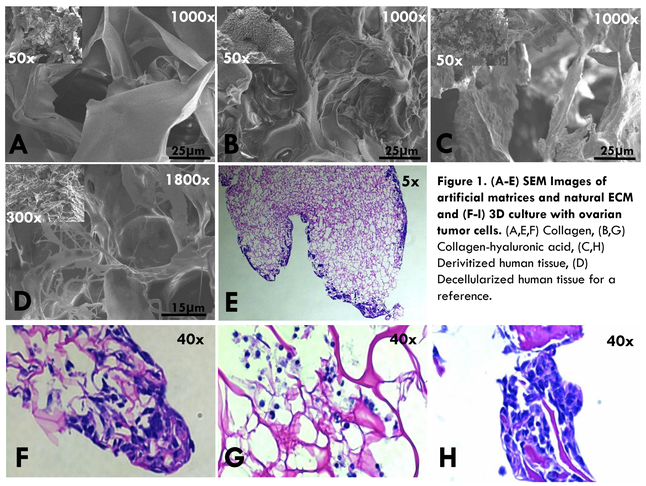


Figure 1. (A-E) SEM Images of artificial matrices and natural ECM and (F-I) 3D culture with ovarian tumor cells. (A,E,F) Collagen, (B,G) Collagen-hyaluronic acid, (C,H) Derivitized human tissue, (D) Decellularized human tissue for a reference.

- Limitations of 3D cell culture include a short culture period and lack of vascularization[1]. Cell migration into scaffolds and membranes is limited by nutrient transport, usually causing them to become encapsulated (Figure 1E).
- The purpose of this study is to mimic the physiologic perfusion of oxygen from blood vessels via micropillar structures of photopolymerized silicone hydrogel in a bioreactor culture environment.
- Silicone hydrogel, a primary constituent in soft contact lenses, was chosen for its biocompatibility and high oxygen diffusivity (60x water).

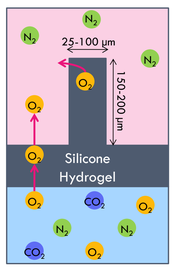


Figure 2. Model of oxygen diffusion through silicone hydrogel micropillars in the bioreactor environment.

Engineering a Microenvironment

- A bioreactor was engineered to mimic the physiological features of the in vivo environment, with multiple design considerations. The environmental conditions of the bioreactor, including gas flow (oxygen, nitrogen and carbon dioxide), pH and dissolved oxygen levels, and circulation rate need to be precisely monitored and controlled.

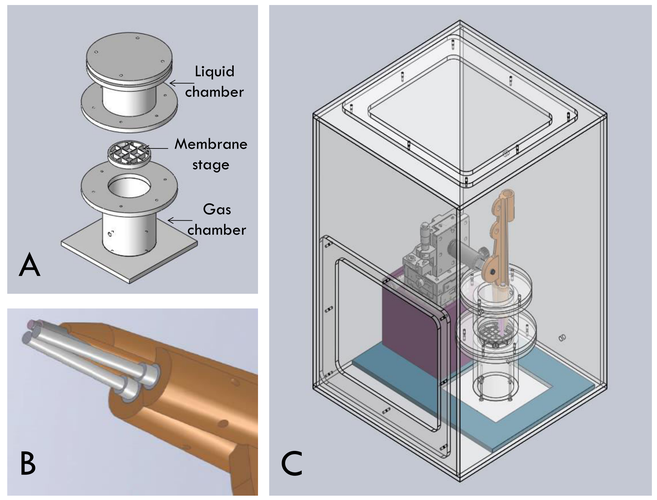


Figure 3. SolidWorks models of the 3D cell culture system. (A) novel bioreactor with mixed gas flowmeter inputs to mimic physiological conditions, (B) microelectrode system designed for sensitive in vitro measurements of oxygen, carbon dioxide and pH, and a (C) container to house the culture system with micrometer driven XYZ stage for precise measurements.

Photomask and Mold Fabrication

- Photolithography was used to create epoxy molds for membrane fabrication. This nanofabrication process involves the transfer of a geometric pattern from a photomask to a light-sensitive photoresist layer on a silicon wafer (Figure 4A).
- To make the photomasks, grid patterns of 25, 50, and 100 µm diameter circles were drawn using AutoCAD and transferred to a quartz surface using a Heidelberg DW66 HeCd Laser writer.

Photolithographic Procedure

Dehydration bake → Spin coat with SU-8 2150 photoresist → Soft bake → Expose against photomask at 400 mJ/cm^2 → Hard bake → Develop in ultrasonic bath

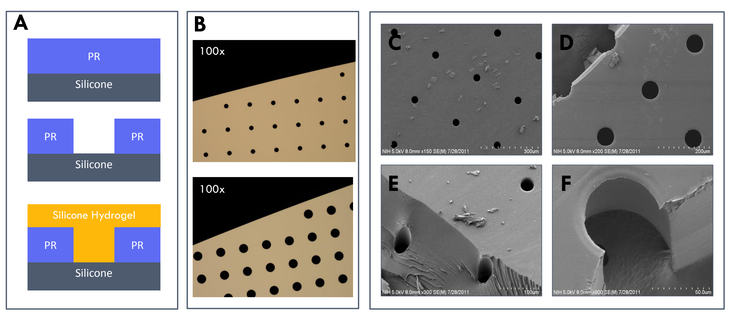
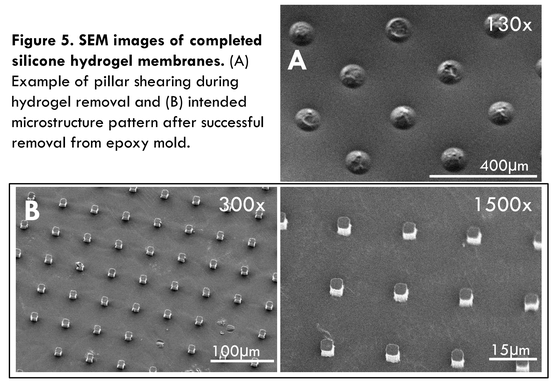


Figure 4. (A) Visual representation of the process design for silicone hydrogel fabrication, (B) completed 50 µm (top) and 100 µm (bottom) photomasks, (C-F) SEM images of epoxy molds used in casting

Silicone Hydrogel Fabrication

- Silicone hydrogel (Dimethyl Acrylamide, Poly(dimethylsiloxane), Ethylene glycol dimethacrylate, N-vinyl-pyrrolidinone, and benzoin methyl ether) was cast onto the epoxy molds and UV cross-linked (365 nm).
- After photopolymerization, the silicone membranes were dried and degassed in a vacuum oven. Samples were sputter coated, and imaged with an Hitachi S-460 instrument.

Figure 5. SEM images of completed silicone hydrogel membranes. (A) Example of pillar shearing during hydrogel removal and (B) intended microstructure pattern after successful removal from epoxy mold.



Conclusions & Future Work

- A bioreactor system was successfully designed and constructed for use in 3D cell culture, whereas hydrogel crosslinking and removal presented the largest obstacles.
  - To facilitate hydrogel removal, a silane release agent was applied to epoxy molds prior to casting via vapor deposition.
  - Crosslinking may be hindered by many factors, including incorrect photoinitiator ratio, and is currently being interpreted
- Future work will involve:
  - Continued optimization of the 3D culture system, specifically silicone hydrogel fabrication and alternative methods for in vitro monitoring of dO_2, pH and dCO_2
  - Histology and gene expression studies on cells cultured under different bioreactor conditions
  - System adaptation for high throughput applications in studies of drug development and resistance

Acknowledgements & References

I sincerely thank my mentors, Tom Pohida and Dr. Michael Gottesman for their exceptional support and guidance. This work would not have been possible without the great inspiration, ideas and enthusiasm of Chandan Das. The cell culture system was constructed at CIT-DCB. Microfabrication was performed at the center for nanoscale technology at NIST and at the Microfabrication and Microfluidics Unit at NIBIB with the help of Dr. Nicole Morgan. Special thanks to the NIBIB-funded BESIP program for supporting this summer of research.

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